MPI RESEARCH
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Analytical Report

PFOA and PFOS Analysis of Deer Muscle and Liver Samples by LC/MS/MS

MPI Report No. L0019346

Revised Report Date: 12/17/09

Testing Laboratory

MPI Research, Inc.
3058 Research Drive
State College, PA 16801

Requester/Project Manager

Dena Haverland
Dalton Utilities
PO BOX 869
Dalton, GA 30722
Phone: 706-529-1010

1 Introduction

Results are reported for the analysis of the 3.5 yr male deer liver sample received at MPI Research from Dalton Utilities. The MPI Research study number assigned to the project is L0019346. Table I lists the target analytes quantitated for the samples.

Table I. Target Analytes for Quantitation

Compound Name	Acronym
Perfluorooctanesulfonate	C8 Sulfonate or PFOS

Note: PFOA and PFOS results for all the muscle and liver samples with the exception of the 3.5yr male deer liver sample are reported in the original report signed on 11/19/09.

2 Sample Receipt

Four samples were received from Dena Haverland at Dalton Utilities for this study. The samples were collected on October 02, 2009. The samples arrived on October 06, 2009 via Fedex and were logged in under MPI Research login number L0019346. The shipment was received frozen on dry ice. The samples were stored frozen at approximately -20°C from receipt until analysis. Chain-of-custody information is presented in Attachment A.

3 Methods - Analytical and Preparatory

3.1 Muscle and Liver Sample Preparation

3.1.1. Weigh 1 g of muscle or liver sample into a 50 mL disposable centrifuge tube and fortify, if appropriate. Add 20 µL of a 50000 ng/mL WIS for a final concentration of 1 ng/mL.

Note: The internal standard was spiked at a higher level to allow for post extraction dilutions to be performed.

3.1.2 Add water to the sample for a final volume of 10 mL. Cap tightly.

3.1.3 Homogenize sample using a tissuemizer for ~1 minute.

3.1.4 Transfer 1 mL of the sample using a disposable pipette into 15 mL disposable centrifuge tubes. Add 5 mL of ACN and shake for ~20 minutes on a wrist action shaker.

3.1.5. Centrifuge tubes at ~3000 rpm for ~ 5 minutes. Carefully decant supernatant into a 50 mL disposable centrifuge tube and add 35 mL of water.

3.1.6 Place the unconditioned SPE columns on the vacuum manifold. Condition the SPE columns by passing ~ 10 mL of methanol through the column followed by ~ 5 mL of water. The washes may be pulled through the SPE column using vacuum at a flow rate of ~1 drop/sec or may be allowed to pass through the column unaided. Discard all washes. Do not allow the column to dry.

3.1.7 Load the sample onto a conditioned SPE column . Discard the eluate. Any analyte residues will be trapped on the SPE column at this point.

3.1.8 Elute with 2 mL of methanol. Collect 2 mL of elute into a graduated 15 mL centrifuge tube.

Note: Post extraction dilutions were prepared in methanol.

3.2 Sample Analysis by LC/MS/MS

In High Pressure Liquid Chromatography (HPLC), an aliquot of extract is injected and passed through a liquid-phase chromatographic column. Based on the affinity of the analyte for the stationary phase in the column relative to the liquid mobile phase, the analyte is retained for a characteristic amount of time. Following HPLC separation, mass spectrometry provides a rapid and accurate means for analyzing a wide range of organic compounds. Molecules are ionized, fragmented, and detected. The ions characteristic of the compounds are observed and quantitated against external calibration standards.

An HP1100 system interfaced to an Applied Biosystems API 4000 LC/MS/MS was used to analyze the sample extracts for quantitation. A gradient elution through a Phenomenex Luna 3µ C8(2) Mercury, 20 x 4.0 mm column was used for separation.

The following gradient was performed:

Mobile Phase (A): 2mM Ammonium Acetate in Water
Mobile Phase (B): Methanol

Time	%A	%B
0.0	90	10
0.5	90	10
2.0	10	90
5.0	10	90
5.1	0	100
6.0	0	100
6.1	90	10
10.0	90	10

The following parameters were used for operation of the mass spectrometer:

Parameter	Setting
Ionization Mode	Electrospray
Polarity	Negative
Transitions Monitored	499→80 (PFOS) 503→80 (Internal Std. ^{13}C PFOS (m+4))
Gas Temperature	450°C

4 Analysis by LCMSMS

4.1 Calibration

For the muscle and liver sample analysis, a 6-point calibration curve was analyzed throughout the analytical sequence for PFOS. The calibration points were prepared at 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 ng/mL (ppb) containing 1.0 ng/mL ^{13}C-PFOS (m+4).

The ratio of the analyte concentration to the IS concentration versus the ratio of the analyte instrument response (area) to the IS response (area) was plotted for each point. Using linear regression with 1/x weighting, the slope, y-intercept and coefficient of determination (r^2) were determined. A calibration curve is acceptable if $r^2 \geq 0.985$.

For the results reported here, calibration criteria were met. The calibration curves are included in the raw data in Attachment C.

4.2 Laboratory Control Spikes

Laboratory control spikes in the analytical set were prepared during each extraction set by adding a known concentration of the analyte to deer muscle and liver controls. Laboratory control spikes are used to assess method accuracy. The laboratory control spikes must show recoveries between 70-130% or the data is rejected. For the results reported here, the laboratory control spikes were within the acceptable range. Laboratory control spike recoveries are given in Attachment B.

4.3 Matrix Spikes

A matrix spike was prepared for each sample by adding a known concentration of the target analyte to a sample. Matrix spikes are used to assess method accuracy in the matrix. The matrix spikes should show recoveries between 70-130%. For the results reported here, the matrix spike was within the acceptable range with the exceptions of:

4.4 Laboratory Duplicates

Each sample was prepared in duplicate and analyzed. Duplicate results are given along with the sample results in Attachment B.

5 Data Summary

Please see Attachment B for a detailed listing of the analytical results. For the muscle and liver samples the results are reported in parts per billion (ng/g) on an as-received basis.

6 Data/Sample Retention

Samples are disposed of 60 days after the report is issued unless otherwise specified by the project manager. All electronic data is archived on retrievable media and hard copy reports are stored in data folders maintained by MPI Research. Hardcopy data is stored for a minimum of five years. The client will be notified 30 days prior to the disposal of hardcopy data.

7 Attachments

7.1 Attachment A: Chain of Custody

7.2 Attachment B: Analytical Results

7.3 Attachment C: Raw Analytical Data for Water

8 Signatures

Mark Neeley, Research Chemist Associate II — Date: 12-17-09

Robert Zhu, Manager, Analytical — Date: 12/17/09

A

MPI RESEARCH

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3058 Research Drive
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Login

Login Group: L0019346

Login #:	19460	Conform COC Sample:	True
Project:	P0005196	Conform COC:	True
Company Name:	Dalton Utilities	Conform Sample:	True
Submitted By:	Dena Haverland	Conform Request:	True
Login Type:	Immediate Receipt of Samples		
Started:	True		
Date Start:	10/27/2009		
Due Date:	11/06/2009		
Login Initiated:	10/27/2009		
Received By:	Ammerman, Mark		
Spread Sample:			
Label:			
MPI SD/PI:	Zhu, Xiang		
Project Title/Type:	PFOA and PFOS Analysis of Animal Muscle and Liver by LC/MS/MS / ROUTINE		
Login Notes:			

Packages / Containers

Package	Carton	Date / Condition	Shipper / ID	Temp. Control/Temp.	Direction / Handled By
K0022042		Received Date: 10/27/09 10:25 Package & Contents Uncompromised	FEDEX 8694 2057 8178	Dry Ice -79.2	RECEIVED Ammerman, Mark

Container #	Gross Weight	pH	Container Type	Preservative	Mfg. Lot	Mfg. ID
C0457624	218.20 g		1/2 gallon ziplock bag	NONE		
C0457625	278.00 g		1/2 gallon ziplock bag	NONE		
C0457626	324.10 g		1 gallon ziploc bag	NONE		
C0457627	994.10 g		1 gallon ziploc bag	NONE		

Samples

Sample ID	Container	Matrix	System	System Matrix	Sample	Date Sampled	Date Due
L0019346-0001	C0457624	SOLID	Deer	Tissue	Deer #6 0.5 yr female-muscle	10/02/2009	11/06/2009
L0019346-0002	C0457626	SOLID	Deer	Liver	Deer #6 0.5 yr female-liver	10/02/2009	11/06/2009
L0019346-0003	C0457625	SOLID	Deer	Tissue	Deer #7 3.5 yr male-muscle	10/02/2009	11/06/2009
L0019346-0004	C0457627	SOLID	Deer	Liver	Deer #7 3.5 yr male-liver	10/02/2009	11/06/2009

Login

Login Reviewed By: _____ Date/Time: 2/16/09 1317

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Sample Submittal

Please fax this form before sending samples.

Please send samples to shipping and receiving:
3048 Research Drive, State College, PA 16801
T: (814) 272-1039 • F: (814) 272-1019

MPI Research Contact: Daniel Wright

Send Report To:

Company: Dalton Utilities

Address: ~~1200 VD Parrott JR Parkway, PO Box 869~~

City, State, ZIP: Dalton, GA 30722-0869

Attention: Dena Haverland

Phone #: 706-529-1010

Fax #: 706-529-1271

Email: dhaverland@dutil.com

Study/Job #:

Signature/Date:

Printed Name:

Turnaround time (TAT) requirements:

Results Due Date: 30 days

Preliminary Results Format: Verbal (Email) Fax

Report Due Date: 30 days

Storage Conditions

Room temperature
Refrigerator
(Freezer)
~~Ultra Low~~ freezer
Desiccated
Lighting required

Stability (°C/%RH):

Stability time period

Safety Information

Special handling:

MSDS attached

Controlled substance:

HAZARDS

Please fill in the diamond HMIS/NFPA (0-4) if appropriate

	Client ID# Description	Lot/ Control #	Amt. Sent/ Weight	# of Bottles	Matrix	Date & Time	Tests Requested
1	Deer #6 0.5 yr Female-Serum		10ml	10	deer	10/2/09 1:08AM	PFOA/PFOS
2	Deer #6 0.5 yr Female-muscle		as requested	1 bag	deer	10/2/09 2:28AM	PFOA/PFOS
3	Deer #6 0.5 yr Female-Liver		Whole	1 bag	deer	10/2/09 2:36AM	PFOA/PFOS
4	Deer #7 3.5 yr Male-Serum		10ml	10	deer	10/2/09 1:45AM	PFOA/PFOS
5	Deer #7 3.5 yr Male-muscle		as requested	1 bag	deer	10/2/09 2:45AM	PFOA/PFOS
6	Deer #7 3.5 yr Male-Liver		Whole	1 bag	deer	10/2/09 2:48AM	PFOA/PFOS
7							
8							
9							
10							

PO #

Relinquished by	Date	Time	Received by	Date	Time
Darrell Kavanaugh	10/5/09	6:57pm	[illegible]	10/6/09	10:25

Notes

"THIS IS AN EXACT COPY OF THE ORIGINAL DOCUMENT"
BY MA DATE 10/27/09

MPI RESEARCH

TEMPORARY SAMPLE STORAGE FORM

To be completed during ExyLIMS Login

Project #: 5196

Login #: L 49346

Initials / Date: MA 10/27/09

One form to be completed for each package

Date / Time Received: 10/26/09 1025

Received By: Mark Ammerman

Shipper: FedEx

Shipper Package ID: 8694 2057 8178

Temperature (deg C) / Thermometer ID: -19.2 / D0001777

Temperature Control Method: dry ice-active

Temporary Storage Location: walk in freezer 11

Condition of sample(s):

✓ Good – Package and contents uncompromised
___ Fair – Package damaged / contents uncompromised
___ Poor – Package and contents compromised

Notes:

FedEx. US Airbill
Express

FedEx Tracking Number 8694 2057 8178

Recipient's Copy

1 From

Date 10/5/09

Sender's Name [redacted] Phone [redacted]

Company [redacted]

Address [redacted]

Dept/Floor/Suite/Room

City [redacted] State [redacted] ZIP [redacted]

2 Your Internal Billing Reference

3 To

Recipient's Name [redacted] Phone [redacted]

Company [redacted]

Recipient's Address [redacted]

We cannot deliver to P.O. boxes or P.O. ZIP codes.

Dept./Floor/Suite/Room

Address [redacted]

To request a package be held at a specific FedEx location, print FedEx address here.

City [redacted] State [redacted] ZIP [redacted]

8694 2057 8178

4a Express Package Service — *Packages up to 150 lbs.*

- [X] FedEx Priority Overnight — Next business morning.* Friday shipments will be delivered on Monday unless SATURDAY Delivery is selected.
- [ ] FedEx Standard Overnight — Next business afternoon.* Saturday Delivery NOT available.
- [ ] FedEx First Overnight — Earliest next business morning delivery to select locations.* Saturday Delivery NOT available.
- [ ] FedEx 2Day — Second business day.* Thursday shipments will be delivered on Monday unless SATURDAY Delivery is selected.
- [ ] FedEx Express Saver — Third business day.* Saturday Delivery NOT available.

FedEx Envelope rate not available. Minimum charge. One-pound rate.

* To most locations.

4b Express Freight Service — *Packages over 150 lbs.*

- [ ] FedEx 1Day Freight* — Next business day.** Friday shipments will be delivered on Monday unless SATURDAY Delivery is selected.
- [ ] FedEx 2Day Freight — Second business day.** Thursday shipments will be delivered on Monday unless SATURDAY Delivery is selected.
- [ ] FedEx 3Day Freight — Third business day.** Saturday Delivery NOT available.

* Call for Confirmation:

** To most locations.

5 Packaging

- [ ] FedEx Envelope*
- [ ] FedEx Pak* — Includes FedEx Small Pak, FedEx Large Pak, and FedEx Sturdy Pak.
- [ ] FedEx Box
- [ ] FedEx Tube
- [X] Other

* Declared value limit $500.

6 Special Handling — Include FedEx address in Section 3.

- [ ] SATURDAY Delivery — Not available for FedEx Standard Overnight, FedEx First Overnight, FedEx Express Saver, or FedEx 2Day Freight.
- [ ] HOLD Weekday at FedEx Location — Not available for FedEx First Overnight.
- [ ] HOLD Saturday at FedEx Location — Available ONLY for FedEx Priority Overnight and FedEx 2Day to select locations.

Does this shipment contain dangerous goods? One box must be checked.

- [X] No
- [ ] Yes — As per attached Shipper's Declaration.
- [ ] Yes — Shipper's Declaration not required.
- [X] Dry Ice — Dry Ice, 9, UN 1845 [redacted] x [redacted] kg
- [ ] Cargo Aircraft Only

Dangerous goods (including dry ice) cannot be shipped in FedEx packaging.

7 Payment *Bill to:* Enter FedEx Acct. No. or Credit Card No. below.

- [X] Sender — Acct. No. in Section 1 will be billed
- [ ] Recipient
- [ ] Third Party
- [ ] Credit Card
- [ ] Cash/Check

Obtain Recip Acct. No. [ ]

Total Packages	Total Weight	Total Declared Value†
1	[redacted]	$ .00

†Our liability is limited to $100 unless you declare a higher value. See back for details.

Credit Card Auth.

8 Residential Delivery Signature Options If you require a signature, check Direct or Indirect.

- [X] No Signature Required — Package may be left without obtaining a signature for delivery.
- [ ] Direct Signature — Someone at recipient's address may sign for delivery. Fee applies.
- [ ] Indirect Signature — If no one is available at recipient's address, someone at a neighboring address may sign for delivery. Fee applies.

520

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BY: [redacted] DATE 10/27/09

MPI RESEARCH

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SAMPLE PROCESSING RECORD

Processing Requested By Employee: Mark Neeley

MPI Research Assigned Project Number: P0005196

MPI Research Assigned Login Number: L0019346

MPI Sample Number	MPI Container Number	Matrix	Equipment Description/Equipment ID Number
L0019346-0001	C0457624	Solid	RobotCoupe / IN0006779
L0019346-0002	C0457625	Solid	RobotCoupe / IN0006779
L0019346-0003	C0457626	Solid	RobotCoupe / IN0006779
L0019346-0004	C0457627	Solid	RobotCoupe / IN0006779

Date Processed: 10/29/2009 Time Started: 14:57 Time Finished: 17:20

Processed By Employee/Employees: Eric Edwards

Processing Instructions/Comments:

B

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Analytical Report

Summary of Fluorochemical Residues in Liver Samples

Sample ID	PFOS Perfluorooctanesulfonate Analyte Found (ng/g, ppb)
Deer # 7 3.5 yr male-liver	2750
Deer # 7 3.5 yr male-liver*	2600

*Laboratory Duplicate
ND = Not detected = Response is below the LOD of 1.0 ng/g (ppb).
NQ = Not quantifiable = Response is between the LOD and the LOQ of 10 ng/g (ppb).

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Recovery Summary of Fluorochemical Residues in Liver Samples

	PFOS			
Sample Description	Amount Spiked (ng/g	Amt Found in Sample (ng/g	Amount Recovered (ng/g	Recovery (%)
LCS A (Data set 112409B) 2000 ng/g	2000	16.5	2340	116
LCS B (Data set 112409B) 2000 ng/g	2000	16.5	2170	108
Deer # 7 3.5 yr male-liver (L19346-4 Spk C, 2000 ng/g Lab Spike)	2000	2750	4770	101

ND = Not detected = Response is below the LOD of 1.0 ng/g.
NQ = Not quantifiable = Response is between the LOD and the LOQ of 10 ng/g.